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JUNE, 1953.

TUBERCULOSIS-LIKE LESIONS IN THE SUBMAXILLARY LYMPH NODES OF PIGS IN QUEENSLAND.

By L. TAMMEMAGI, B.V.Sc., Dr. med. vet., Animal Health Station, Yeerongpilly.

SUMMARY.

Sixty-five diseased submaxillary lymph nodes of pigs were studied macroscopically, bacteriologically, histologically and by animal inoculation. In addition, 75 normal nodes were examined bacteriologically and 20 of them also histologically.

Corynebacterium equi was recovered from 36 of 38 nodes with tuberculosis-like lesions. It was the sole organism in 14 of these nodes and is regarded as being responsible for the pseudo-tuberculous lesions. No evidence was found that any of the miscellaneous other bacteria frequently associated with C. equi, or any migrating parasitic larvae, were responsible.

Of the 15 nodes diagnosed by meat inspectors as tuberculous, tubercle bacilli were recovered from 8. The strains were all of bovine type. C. equi was isolated from 9 nodes in this group, including 3 nodes in which C. equi was associated with tubercle bacilli.

The lesions due to C. equi are distinguished from tuberculosis in that the corynebacterial nodules are spherical or oval in shape, encapsulated and readily enucleated. Histological examination is of little value in differentiating the two conditions, but a dense, though often rather narrow, fibrous capsule around C. equi lesions is a fairly constant distinguishing histological feature.

C. equi was found also in 22 of 75 normal and in 2 of 12 nodes showing a net-like mottling in a restricted area of otherwise normal lymph node tissue. The net-like pattern was shown to be due in part to proliferation of the trabeculae and probably also to distended sinuses. The condition apparently represents a healing process from a previous local bacterial infection.

The partial acid-fastness of C. equi was confirmed. About 22% of the 68 strains studied did not hydrolyze urea and 4% did not produce H_2S. Marked eosinophilia in the lymph nodes in corynebacterial infection is apparently of no significance, as this is seen also in normal lymph nodes.

INTRODUCTION.

The recovery by Holth and Amundsen (1936) in Norway of a gram-positive and partly acid-fast "coccobacillus" from presumably tuberculous "cervical" lymph nodes of pigs, but from which no tubercle bacilli were recovered, has given an impetus to further study on the occurrence of tuberculosis-like lesions in pigs' lymph nodes. In Scandinavia, Plum (1938), Jesperson (1938), Magnusson (1938) and others confirmed the frequent association of this "Holth bacillus" with tuberculosis-like lesions in the submaxillary nodes of pigs. Bendixen and Jepsen (1938) identified this organism as *Corynebacterium equi*, a diphtheroid which was first described by Magnusson (1923) as a cause of specific pneumonia and pyogenic lesions in foals.

C. equi has been subsequently recovered in many other countries. McDonald (1942) and Woodroofe (1950) isolated it from tuberculosis-like lesions in the submaxillary nodes of pigs slaughtered in South Australia. A study was therefore undertaken to determine (*a*) the cause of the lesions resembling tuberculosis which frequently have been observed in the submaxillary nodes of pigs slaughtered in Queensland and usually referred to by meat inspectors as "worm" nodules, and (*b*) whether these pseudo-tuberculous lesions have any characteristics which might facilitate their differentiation from true tuberculosis.

MATERIAL AND METHODS.

Source of Material.

The specimens examined in this study were the submaxillary lymph nodes of pigs slaughtered at the Doboy bacon factory in Brisbane during the months from March to August, 1952.

Specimens were collected on 17 occasions, usually at weekly intervals. The carcases from which the specimens were taken were examined for any gross lesions. In routine inspection the left node was incised first. If it was found to be diseased, it and the intact right node were placed in separate sterile jars and examined in the laboratory. If the right node showed lesions similar to those in the left node it was used for further study. The left nodes were discarded. A record was made of the meat inspector's diagnosis, source and age of the pig, and the presence or absence of kidney worm (*Stephanurus dentatus*) infestation.

Examination at the Laboratory.

Each of the specimens (right node) was freed from surrounding tissue, plunged into boiling water for 7-10 seconds, and kept in the refrigerator in a separate petri dish until all specimens were dealt with, which was usually within 4-6 hours after collecting the first specimens at the abattoir. Each node was then sectioned under aseptic precautions and examined for gross abnormalities. If there were no lesions or if the lesions differed from those in the left, the node was discarded. In the case of visible lesions, their size, shape, number and distribution, colour, consistency, presence of a capsule and ease of enucleation were recorded.

Bacteriological Methods.

A portion of each node containing lesions was made into a suspension by grinding with sterile sand and gradually adding saline solution up to 5 ml. Following sedimentation of the larger particles, loopfuls of the supernatant fluid were transferred on to a 10% sheep's blood-agar-plate, a Loeffler's serum slope and at least two of each of Dorset's and Lowenstein-Jensen egg media. The blood-agar-plates were examined after 24 hours and 48 hours of incubation at 37°C. Loeffler's serum slopes were examined for *C. equi* after 48 hours' incubation and then at weekly intervals for 4-6 weeks. The egg media were examined for growth of tubercle bacilli at weekly intervals for 6-8 weeks, and then discarded if no growth appeared after this time.

To prevent the growth of contaminants, which sometimes interfered with the detection of tuberculous colonies, a modified technique was applied in a later group of 15 specimens. After dealing with the nodes in the usual way, the content from one or several encapsulated lesions was shelled out aseptically and ground up with a little sterile sand and approximately 0.5 ml. of saline solution to a uniform milky suspension. A loopful of this suspension was spread over a blood-agar-plate and a Loeffler's serum slope for detection of *C. equi,* and the remainder of the suspension was then diluted to 4 ml. with more saline solution, 2 ml. of which was used to inoculate guinea pigs. The remaining 2 ml. was divided equally into two sterile centrifuge tubes, each of which contained 1 ml. of sterile 5% oxalic acid solution. Where the lesions were extensive and in masses, so that no single nodules could be separated, whole lymph node tissue was ground up to make the suspension and dealt with in a similar manner. The centrifuge tubes were incubated at 37°C. for 30 minutes with frequent shaking; 10 ml. of sterile saline solution was then added to each tube, the tube plugged with cotton-wool and centrifuged for 30 minutes. All but 0.5 ml. of the supernatant fluid was discarded, and the sediment thoroughly mixed with the fluid which remained by means of a platinum loop. With a capillary pipette all the suspension from both tubes was then spread over four Dorset's and four Lowenstein-Jensen egg media.

In addition, in cases where normal tissue was available in the diseased lymph nodes, several sections of such tissue, surrounding but remote from the lesions, were smeared directly over the surface of a second blood-agar-plate.

The presumably normal lymph nodes, showing no visible lesions, were examined for the presence of *C. equi* or other bacteria but not for tubercle bacilli. For this purpose cut sections were directly smeared over blood-agar-plates, as this method in a special trial gave a higher incidence of *C. equi* growth than did ground-up suspension of the same node.

Guinea Pig Inoculation.

Two animals were each inoculated subcutaneously into the thigh with 1 ml. of untreated (no oxalic acid) emulsified lymph node tissue or with the suspension of the content of the nodules. One animal from each pair was killed about a month later, and the other after 6-8 weeks. Tuberculosis was

diagnosed usually by the gross lesions, but smears and cultures were made from any abnormalities in the regional lymph nodes, spleen or liver. In case of negative post-mortem findings the spleen was cultured to recover any tubercle bacilli of the avian type, which as a rule does not produce gross lesions in guinea pigs, but which may be cultured from spleens of normal appearance. No animal inoculation was done with normal specimens.

At autopsy, blood was tested for *Brucella* agglutinins.

Histological Methods.

Selected portions of each node, contaning half of a lesion or more, were fixed in 10% formol-saline solution. Sections were stained with hematoxylin and eosin, and by Ziehl-Neelsen using methylene blue as counterstain.

Of the normal specimens only 20 were studied histologically.

RESULTS.

Altogether, 140 lymph nodes (right side) were selected for examination. All of these were found in the laboratory to have the same gross appearance as their left side counterparts, which had been classified in the abattoir by the meat inspectors as follows:—15 as tuberculous, 38 as pseudo-tuberculous, 12 as "mottled" and 75 as normal lymph nodes. This classification, which was done by two inspectors, was not altered during the study, though for some specimens presumed tuberculous, close examination in the laboratory raised a slight doubt as to their tuberculous nature.

Tuberculous Nodes. Pathology.

Of the 15 specimens, 7 on closer study were recognized as being indisputably tuberculous (referred to as 1st sub-group). They were characterized by marked enlargement and fibrosis of the nodes ("potatoes") with extensive masses of caseous or caseo-calcareous material, the whole usually surrounded by a strong fibrous capsule. The lesion itself was usually divided by interlacing fibrous strands, and occupied either the whole or the greater portion of the node. In six instances these lesions were the only ones detected in the animal at the abattoir, but in the seventh case (No. 127) characteristic tuberculous lesions were found also in the liver and bronchial nodes.

In the remaining 8 specimens (sub-group 2), where a doubt arose on the true character of the lesions, there was no enlargement and fibrosis, and instead of a diffuse caseation, few to multiple discrete lesions were present, ranging from the size of a pinpoint up to 2-3 mm. across, and except in one case (No. 130) apparently not encapsulated. The nodules, spherical or oval in shape, showed a cream-coloured or yellowish discoloration, and their caseous or gritty content was as a rule enucleated only with difficulty. Only in few instances could the larger nodules be enucleated more readily, while the smaller lesions in the same specimens were comparatively fast in the tissue. In one case (No. 1), in addition to the discrete non-capsulated and gritty nodules, tuberculous lesions were seen also in the liver and bronchial nodes.

Pseudo-tuberculous Nodes.

The 38 nodes in this group were classified by the inspectors as "non-tuberculous" on the grounds that no small lesions of pinpoint size, a feature which was thought by them to indicate a tuberculous infection, were observed when the nodes were incised and examined. They contained, however, larger discrete nodules, usually enclosed by a visible capsule, from which the contents were readily enucleated. During this study, when the specimens were collected, the incidence of these apparently non-tuberculous nodules in the submaxillary nodes of pigs slaughtered at the Doboy bacon factory was recorded; it was found to be between 3.5% and 7%, while only in one of every 10-20 of such animals did the gross lesions suggest tuberculosis. Often several pigs from the same farm showed these discrete encapsulated lesions.

Closer examination, however, revealed that though nodules were usually more or less uniform in size, ranging from 1 mm. to 4 mm., rarely up to 5 mm., in diameter, small lesions of pinpoint size or even smaller were often present, but were detected only microscopically.

The number of lesions in any one node varied as follows:—5 nodes showed 1 lesion, 15 nodes 2-5, 9 nodes 6-10, and another 9 nodes 11-20 or more nodules. In three nodes, in addition to the discrete nodules, there were one or two pear-shaped conglomerates, composed of 5-8 single nodules surrounded by a common capsule. Each single nodule within the conglomerate was distinct and easily separated. The lesions were usually scattered at random in the lymph node tissue; in some they were concentrated in a certain portion or towards one pole of the node. Where the lesions were superficial, their presence and contour were evident before incision.

The colour of the lesions ranged from off-white in 21% of cases to cream-coloured in 60% and slightly yellowish in 19%. Two specimens showed a slight greenish tinge.

In one specimen (No. 43), which contained several lesions from pinpoint size up to 3 mm. in diameter, the shape of the larger nodules was irregularly angular, the smaller being elongated or comma-shaped, and thus quite different from the usual spherical or oval-shaped nodules of the other specimens in this group. As no capsule formation could be distinguished around these lesions and enucleation was difficult, a doubt arose as to the correct classification of this specimen by the inspector. However, the examination at the abattoir was deliberately superficial. The left submaxillary node was normal and the lesions described here were revealed in the opposite node after a single incision. The enucleation test was omitted to preserve the specimen, and the lymph node was tentatively judged as non-tuberculous because of the discrete nature of the lesions seen on the cut surface. Later, tubercle bacilli were recovered from this specimen by guinea pig inoculation.

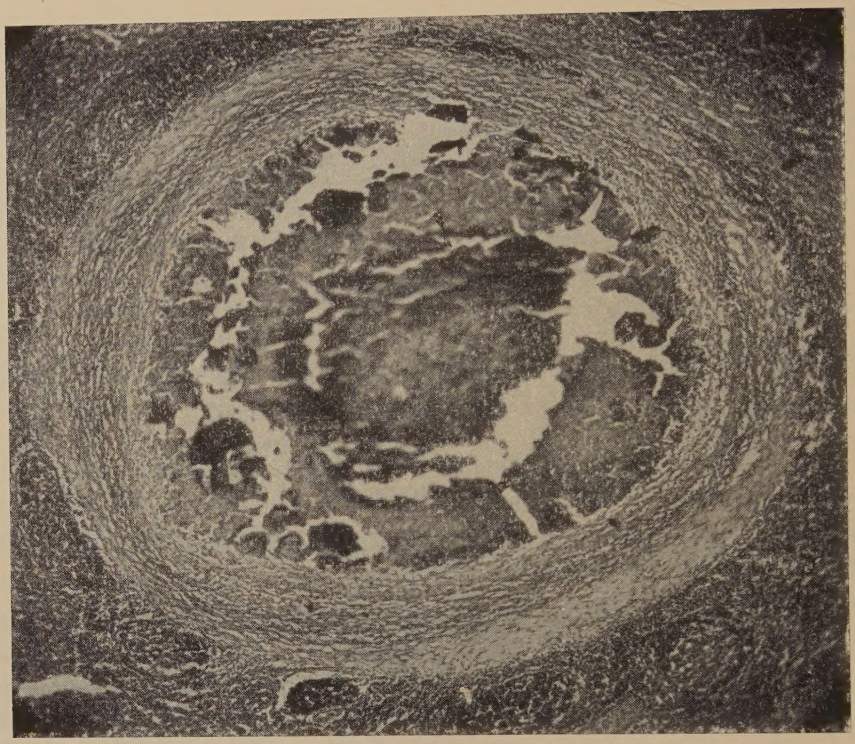

Fig. 1.

Tuberculosis-like Lesion in the Submaxillary Node of a Pig. The intermediate zone between the peripheral dense (in this case rather thin and not complete) fibrous capsule and the central caseating area is a granulation tissue with a circular arrangement of the fibres, in which are enmeshed lymphocytes, macrophages and a few epithelioid cells. *C. equi* was isolated from this specimen. (x45).

A ready enucleation of the lesions and the presence of a capsule, criteria used as the main characteristics for classification of the lesions as non-tuberculous by the inspectors, were features given careful consideration when the specimens were studied in the laboratory. By cutting with a sharp scalpel, a definite layer, suggestive of a capsule, was observed in lesions over 2 mm. in diameter. This layer was absent in only one specimen (No. 43). The thickness of this layer varied from about 1 mm. down to a small fraction of a millimeter. The content of these nodules was readily enucleated with the point of a knife or by slight manual pressure, and a smooth or slightly pitted depression was left behind. The capsule itself in some instances seemed to have a variable thickness.

The other nodules in the same nodes, noticeably the smaller ones less than 1.5 mm. across, often did not indicate a capsule to the naked eye, but the content usually was readily enucleated. In the very small lesions, from 1 mm. down to pinpoint size, the enucleation usually followed only after using strong pressure.

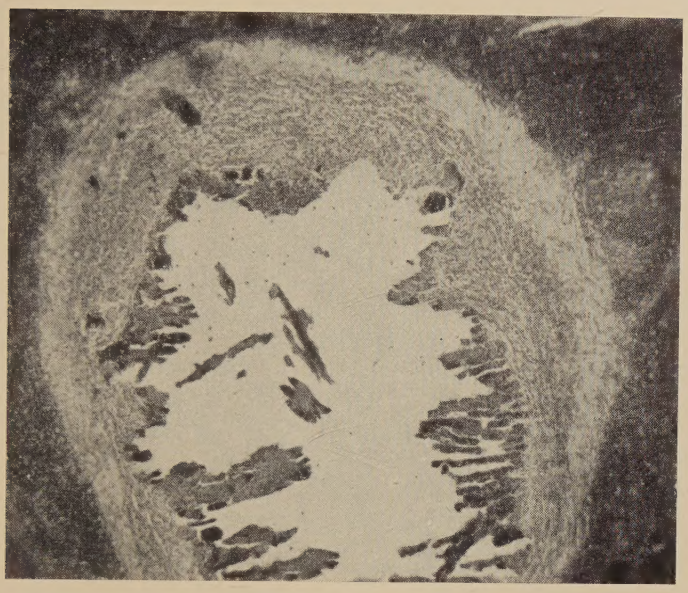

Fig. 2.

Encapsulated Corynebacterial Lesion in which the caseating centre is more irregular in Shape than in Fig. 1. Most of the centre was lost in preparing the tissue for histologic sections. The intermediate zone shows, instead of a circular and regular arrangement of the fibres, a more irregular pattern, resembling to some extent tuberculous granulation tissue. One giant cell can be seen embedded in this granulation tissue. Several small calcified foci are present in the necrotic centre and also in the granulation tissue zone. (x60).

The content of the lesions in general showed great variation in consistency, but as a rule, lesions of about equal size in the same node were more or less uniform in this respect. The very small lesions, from pinpoint size up to 1 mm. across, were often dry or gritty. In the larger nodules, the consistency varied from soft caseous or putty-like to firm caseous or dry, giving a feeling of calcium deposition. Calcification was never complete, at least in the larger nodules, but even the most gritty of the smaller nodules was compressible between glass slides, disintegrating into mortar-like granular particles. In a few specimens, the content of the larger lesions had a somewhat firm elastic character, and would slip away when compressed under a slide. The content of such nodules was easily expressed by applying slight pressure. In general, a distinct classification of the lesions according to their consistency was never successful, because considerable variation in consistency often occurred in the nodules of the same lymph node. Entirely soft toothpaste-like consistency was seen in three or four specimens containing also nodules with a firmer material.

In four specimens, some of the larger lesions, 3-4 mm. across and with a putty-like or firm caseous or elastic material, seemed to be composed of several more or less distinct layers. The content as a whole was easily enucleated from the surrounding capsule, but did not fall into layers when separation was

Fig. 3.

An Encapsulated Corynebacterial lesion which has a striking resemblance to a Conglomerate Tubercle. Numerous giant and epithelioid cells, enmeshed in the granulation tissue, emphasize the resemblance to tuberculosis. *C. equi*, but no tubercle bacilli, was recovered. (x60).

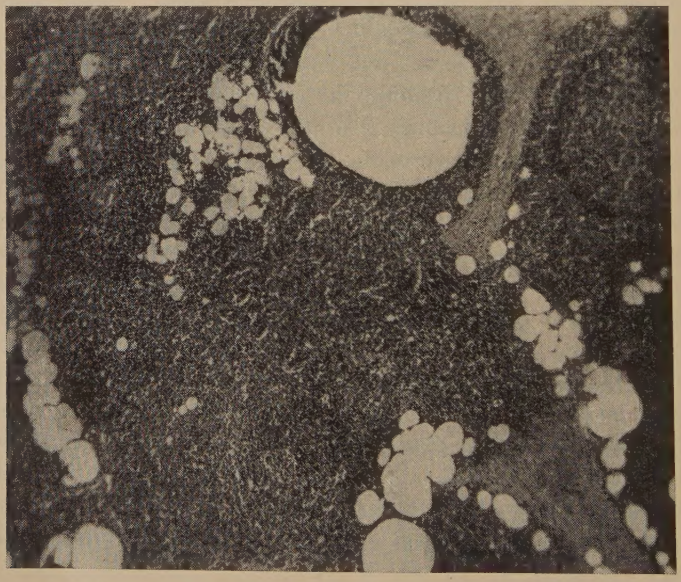

Fig. 4.

Section of a Mottled Lymph Node. Apart from a proliferation of the fibrous strands of the trabeculae, a large number of vaculoes is present in the lymphoid tissue. Many of the smaller vacuoles are in confluence in clusters or in rows, but the largest vacuoles are seen in the primary nodules, where they have replaced the germinal centres. (x60).

B

Table 1.

BACTERIOLOGICAL RESULTS.

Group.	Specimens.*	Tubercle bacilli and *C. equi* recovered.										Other organisms recovered.													
		M. tuberculosis. Total.		*C. equi.* Total.		*M. tuberculosis.* Alone. †		*C. equi* Alone. †		Both *M. tuberculosis and C. equi.* †		Unidentified Corynebacteria.		Saprophytic Mycobacteria.		Proteus.		Haemolytic Streptococci.		Micrococci.		Staphylococci.		Gram-negative Organisms.		Unspecified Organisms. ‡	
		Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%	Number.	%
Tuberculous	15	8	53·3	9	60·0	5	33·3	6	40·0	3	20·0	1	6·6	3	20·0	..	..	6	40·0	8	53·3	2	13·3	2	13·3	..	..
Pseudo-tuberculous ..	38	1	2·6	36	94·7	..	..	35	92·1	1	2·6	..	..	4	10·5	1	2·6	12	31·6	5	31·6	5	13·1	6	15·8	..	..
"Mottled"	12	..	..	2	16·6	..	..	2	16·6	..	..	..	..	..	..	..	..	7	58·3	5	41·6	3	25·0	3	25·0	..	..
Normal	75	..	..	22	29·3	..	..	22	29·3	..	..	..	..	..	..	3	4·0	38	50·6	?	?	?	?	?	?	54	72·0

* = Each specimen represents one animal.

† = Ignoring all "other organisms" for this purpose.

‡ = Includes micrococci, staphylococci and gram-negative organisms.

attempted. It only became evident on close examination that the central portion contained a somewhat drier or granular, mortar-like material, whereas the peripheral layers were more smooth and uniform in texture.

"Mottled" Lymph Nodes.

This mottling, which consisted of small areas of fine, whitish, net-like interwoven lines of variable thickness, 0.1-0.5 mm. in diameter, was met at the abattoir quite often in submaxillary nodes which otherwise looked fairly normal. As a rule it occupied one or several separate lobules of the lymph node, but was usually restricted to the periphery of the tissue. It was readily seen through the lymph node capsule, but became more distinct in cut sections. It occurred in pigs 5-9 months old, which was the usual age of the animals brought for slaughtering. In the younger pigs this mottling was usually less developed than in older ones, in which sometimes a large portion of the node was involved. The greyish-white cut surface then resembled to some degree a primary tuberculous caseation.

Twelve such nodes were studied bacteriologically and histologically.

Normal Lymph Nodes.

The cut surfaces of the 75 specimens studied were in general of normal appearance. However, 16 nodes showed a slight to rather definite injection with pinpoint–to pinhead–sized haemorrhages throughout or in portions only of the lymph node tissue.

Bacteriology.

The results are summarized in Table 1.

Mycobacterium tuberculosis.

Tubercle bacilli were recovered from 8 of the 15 specimens classed as tuberculous by the inspectors, and from 1 of the 38 specimens classified as non-tuberculous. No tubercle bacilli were isolated from "mottled" nodes. Normal lymph nodes were not examined for tubercle bacilli.

Table 2.

RECOVERY OF TUBERCLE BACILLI AND *C. equi* FROM DISEASED LYMPH NODES.

Group.	Specimens.	Tubercle bacilli Recovered.	*C. equi* Recovered.
Tuberculous—			
1st sub-group	7	7	3
2nd sub-group.. ..	8	1	6
Total	15	8	9
Pseudo-tuberculous ..	38	1	36

In the tuberculous group (Table 2), tubercle bacilli were recovered from all the seven specimens placed in the first sub-group, but in the second sub-group from only one of the specimens (No. 1), which showed gross lesions also in the liver and bronchial nodes.

Ignoring for the present the various other organisms, such as streptococci and micrococci, which were frequently found, then out of these eight bacteriologically confirmed tuberculous cases, *M. tuberculosis* was isolated alone in five instances, while in the remaining three *C. equi* was recovered as well.

Tubercle bacilli were isolated once only in the pseudo-tuberculous group. This was No. 43, which had shown the atypical, irregularly shaped lesions with no capsule. The submaxillary node of another pig (No. 42) from the same farm revealed only *C. equi*, although the lymph node in this pig contained 20 or more lesions of variable size, some in conglomerates. Capsule formation was distinct in the larger nodules, and their shape typically spherical or oval with contents readily enucleated.

Appearance of the colonies obtained either directly or by culture from the lesions produced in guinea pigs on Dorset's and Lowenstein-Jensen egg media was suggestive of the bovine type of *M. tuberculosis*.

Corynebacterium equi.

Description of the Strains Recovered.—Identification of the *C. equi* strains recovered in this study was based upon the morphology, staining properties, cultural characteristics and biochemical reactions presented in Bergey's Manual of Determinative Bacteriology (1948) and by Morse (1950).

The pleomorphism of the organism was clearly evident. In nutrient broth the bacillary form was predominant, with frequent occurrence of the branching or V arrangement of the rods. On solid media usually the coccoid form was represented, often in short chains, giving the impression of a micrococcus.

Good growth on blood-agar-plates followed within 48 hours of incubation, showing the characteristic large (up to 8 mm. in diameter), smooth, glistening, semi-transparent and slightly raised mucous colonies. A slight pinkish discolouration was apparent after longer incubation. No haemolysis occurred. On Loeffler's serum slopes the usually confluent growth showed this pinkish colour within two days of incubation. On the egg media the initial growth was smooth and salmon pink after two days, but it acquired a finely rugose or coarse pebbled surface when incubated longer, the colour gradually deepening to brick red.

None of the strains produced acid or gas from glucose, lactose, sucrose, maltose or mannitol. Gelatin was not liquefied, and Loeffler's serum and milk agar slopes were not digested. The ability to hydrolyze urea in Christensen medium was variable. The majority of strains were active hydrolyzers, but many were less active and 15 strains of 68 (22%) were inactive in this respect, even after incubating for 12 days. Similarly, the production of H_2S was variable, and approximately 4% of the strains were inactive in this respect.

The partial acid-fastness of *C. equi* was readily demonstrated in all strains by the presence of pinkish or red stained spheres or ovals in films treated for 5 minutes with 25% HCl solution during staining by Ziehl-Neelsen.

No acid-fast forms were seen during the first 24 hours of incubation, either in liquid or on solid media. The first acid-fast forms were demonstrated after 48 hours, but their number varied greatly. In nutrient broth usually only very few were present in a whole loopful of the fluid culture, but in some strains up to 20% of the organisms visible in a microscopic field showed acid-fastness. On solid media their number was usually higher, but still varied considerably. Some strains showed only very few, but in others their number was roughly from 1% up to 10% or even to 50%. Comparing different solid media, it was found that on the Lowenstein-Jensen media their incidence was higher than on nutrient agar slopes, while some strains on agar showed initially a higher incidence than on egg media, but after a peak was reached on the third or fourth day, the number dropped, and a week later few or no acid-fast forms at all were detected. On egg media, the peak was usually reached in the second week, and high incidence was maintained for weeks, though strains often varied considerably among themselves. In fluid media the acid-fast forms disappeared usually after six weeks.

The degree of the acid-fastness was not uniform, as often in the same film forms were found which had shown a lower resistance to the decolorizing agent, as judged by their pale or only very slight pinkish colour. In others the beginning of a probably "acid-fast phase" was indicated by the appearance of small red dots in one or both poles of the otherwise bluish stained oval, when methylene blue was used as counter-stain.

In addition to the typical predominant oval or coccoid forms, long or short plump rods, usually with one end tapered, or pear- or comma-shaped acid-fast forms were also observed. They were encountered in broth and egg media, but not in nutrient agar slopes. The earliest appearance was after three days of incubation, but usually they appeared from the second week onward with increasing frequency. However, their number was generally low, and the oval or coccoid forms always predominated.

That all these multishaped acid-fast forms were really *C. equi* was proved to some extent by the fact that single red-stained ovals or spheres were often found within unbroken chains of organisms of the same shape. Further, the appearance of the growth in subcultures on blood-agar-plates and egg media, and the examination of the colonies and films from the latter, did not reveal tubercle bacilli or contamination of any kind, even after prolonged incubation. In four cases 1 ml. of the broth cultures containing these multi-shaped acid-fast forms was inoculated subcutaneously into guinea pigs, but no tuberculous lesions were produced.

Recovery of C. equi.—Nine strains of *C. equi* were recovered from the tuberculous group (60%) and 36 strains from the pseudo-tuberculous group (94·7%). No growth was obtained from one specimen of the pseudo-tuberculous group, but typical acid-fast forms of this organism were seen in direct smear from the calcified nodule material. Two strains were obtained from the "mottled", and 22 strains (29·3%) from apparently normal lymph nodes.

In the group classed as tuberculous by the inspectors (15 nodes), *C. equi* was the only isolation in six cases. All of these were in the second sub-group. *C. equi* was also recovered in three specimens in the first sub-group together

with *M. tuberculosis*. From two cases in the second sub-group no *C. equi* was recovered; in one of these (No. 1) tubercle bacilli were isolated alone, and in the other (No. 26) an unidentified non-haemolytic corynebacterium was recovered together with a non-pathogenic saprophytic mycobacterium.

In the pseudo-tuberculous group only two specimens failed to yield *C. equi*. One was sterile, but from the second, micrococci were cultivated.

In the majority of cases, *C. equi* was isolated by using as inoculum a suspension of the diseased lymph node tissue. In nine cases, however, where the content of the nodules was used for culturing, *C. equi* was recovered from all nine as compared with five recoveries only when in the same specimens the surrounding normal tissue away from the lesions was used for plating. In all these nine cases, *C. equi* was the sole organism recovered from the content of the lesions. By plating of the surrounding tissue of the same nodes, haemolytic streptococci and/or micrococci as well were isolated in six instances.

Saprophytic Non-pathogenic Mycobacteria.

These were recovered in direct culture from seven specimens—three from the tuberculous (2nd sub-group) and four from the pseudo-tuberculous specimens. None of these strains was pathogenic to guinea pigs when 1 ml. of a heavy suspension of pure culture was inoculated subcutaneously. Only one strain produced an abscess at the site of injection, but it was not recovered from the regional lymph node, liver or spleen, which showed no gross changes. These strains showed a rapid and profuse growth on nutrient agar and on egg media. Some strains produced a profuse growth after 2–3 days of incubation, but the others showed a definite growth from the fifth or sixth day onwards. Three strains produced an orange-coloured, smooth, confluent growth, and were later identified as *Mycobacterium phlei,* using the technique described by Gordon (1937). The remaining strains were not identified, but some appeared to belong to group IIIb in Gordon's tabulation (*Mycobacterium* sp.).

Miscellaneous Organisms.

In addition to the organisms already dealt with, various other bacterial types, as shown in Table 3, were recovered. The gram-negative organisms were mostly coliforms; pasteurella was present in one specimen in the pseudo-tuberculous group.

Table 3.

Miscellaneous Bacteria Recovered from the Specimens Examined.

Group.	Number of Specimens.	Haemolytic Strepto-cocci.	Micrococci.	Staphylo-cocci.	Gram-Negative Organisms.	Proteus.	Sterile.
Tuberculous ..	15	6	8	2	2	..	..
Pseudo-tuberculous	38	12	12	5	6	1	1
Mottled	12	7	5	3	3	..	..
Normal, total ..	75	38	?	?	?	3	15
One batch of 20 normal lymph nodes	20	8	6	..	14	1	6

The general finding was that either one only or two or more different organisms in any combination were represented simultaneously either alone or in association with tubercle bacilli and/or *C. equi* (see Table 4).

Table 4.

Incidence of Various Combinations of the Miscellaneous Bacteria (Tubercle Bacilli and *C. equi* Excluded).

Group.	One kind of organism present.	Two different organisms present.	Three different organisms present.
	Per cent.	Per cent.	Per cent.
Tuberculous	33·3	53·3	..
Pseudo-tuberculous ..	31·6	26·3	2·6
Mottled	50·0	50·0	..

In the pseudo-tuberculous group one node was sterile and in 14 cases out of 36 specimens (approximately 40%) *C. equi* was found alone.

In the group of normal lymph nodes, no detailed examinations were completed for all the specimens, but 15 nodes (20%) were sterile. In the others, two, three, or more organisms were often recovered simultaneously. In one node with small haemorrhages, five different organisms, including *C. equi,* were isolated. There is, however, the possibility that some airborne contamination may have occurred during the manipulation of the material. On two occasions, when blood-agar-plates were kept uncovered for half an hour in the abattoir and at the laboratory bench and then incubated, various bacteria grew, but no haemolytic streptococci, though the latter were recovered from the material dealt with on the same day. This suggests that streptococci at least were definitely associated with the lymph nodes examined. Of the haemolytic streptococci recovered from the tuberculous and pseudo-tuberculous specimens, eight strains were studied biochemically and serologically. Three of these belonged to Lancefield group C. Four of the others appeared biochemically to be *Str. bovis,* and one *Str. faecalis.*

Bacteriology of the Haemorrhagic Nodes.

In 16 cases small haemorrhages were seen in otherwise normal lymph nodes. The condition was also seen in three nodes in the tuberculous, and in two nodes in each of the pseudo-tuberculous and "mottled" groups, but no definite correlation was found between the bacterial flora isolated and those haemorrhages. In some cases such nodes were sterile.

Brucellosis.

No *Brucella* agglutinins were demonstrated in any of the 140 guinea pigs inoculated with material from the tuberculous, pseudo-tuberculous or mottled lymph nodes, and examined 4–8 weeks later.

Histology.

Tuberculous Nodes.

Histologically advanced tuberculosis was noted in all those lymph nodes in this group which had yielded tubercle bacilli. Though no tubercle bacilli were detected in sections stained by Ziehl-Neelsen, the histology was typical of tuberculosis.

In the advanced cases in this group (sub-group 1, Table 2) the normal lymph node elements were to a large extent replaced by tuberculogenic granulation tissue, in which the typical epithelioid-cell-tubercles were present in great numbers either singly or more commonly united into larger conglomerates. Frequently the whole conglomerate had undergone caseous necrosis, revealing then to the naked eye angular or irregularly shaped cores of 2-4 mm. across. In many specimens the necrotic and caseating masses were fused together into larger caseous areas, in which multiple calcified foci were scattered. Considerable quantities of fibrous tissue in the form of strong interlacing bands were present in all specimens examined. It was often so arranged as to suggest in places the presence of a capsule. On microscopic examination this was actually thought to be so in specimen 127. However, a true fibrous capsule as described later for the typical pseudo-tuberculous lesions was not evident, though frequently capsule formation was suggested by the presence of a few layers of loosely arranged fibres around the periphery of the tubercles. Additionally, capsule formation was suggested by the reticular fibres of the tuberculogenic granulation tissue itself. Usually the latter had a rather irregular pattern, but showed a more circular and regular arrangement of the fibres when enclosing the tuberculous foci, probably due to compression by the expanding tubercles.

In specimen 43, the single specimen in the pseudo-tuberculous group from which tubercle bacilli were isolated, no fibrosis of the lymph node tissue was present and no tuberculogenic granulation tissue was detected around the discrete multiform lesions. The latter were of variable size and not sharply outlined against the surrounding lymphoid tissue. In fact, a tumour-like infiltration of the normal lymphoid tissue with epithelioid-type cells in the periphery of the irregular necrotic lesions was the most noticeable feature. Multiple microscopic islands of epithelioid cells, often comprised of only few odd cells, were dispersed in the lymphoid tissue, but were found in greater concentration especially around or in the vicinity of the larger lesions. The central portions of the lesions, apparently formed by fusion of smaller units, showed a coagulative necrosis with multiple separate aggregations of neutrophils.

In the remaining lymph nodes in the tuberculous group, none of which had revealed viable tubercle bacilli (sub-group 2, Table 2), but all of which had yielded corynebacteria, the histology, though simulating tuberculosis to some extent, was somewhat different and resembled the histology of the pseudo-tuberculous lesions described below.

Pseudo-tuberculous Nodes.

The general histological picture of the pseudo-tuberculous lesions, which mostly had yielded corynebacteria but, except for case No. 43 noted above, no viable tubercle bacilli, was of a spherical or oval-shaped discrete nodule with a caseating centre and a definite fibrous capsule. This capsule, usually very dense though sometimes rather thin and not always complete, was charactertistic for most of the lesions, even the smallest where macroscopically no capsule was detected. The capsule was present also around the lesions in the specimens

of the 2nd sub-group (Table 2) of the tuberculous group, which macroscopically had not indicated the presence of a capsule. However, it became apparent that the peripheral layer of the lesions, which often on macroscopic examination was diagnosed as the capsule, was not always the true or fibrous capsule alone. The latter was in many instances so thin as to render its recognition very unlikely by the naked eye. In fact, a variable portion of granulation tissue, representing the intermediate zone between the fibrous capsule and the necrotic or caseating central mass, was nearly always seen to adhere to the capsule proper when the content was removed, so increasing the total thickness of this capsule to visible proportions. In two specimens, in addition to the typical encapsulated lesions, one nodule was found in each specimen where a dense fibrous capsule was absent. The respective nodules were about 4 mm. in diameter. Around the centrally situated, fused, calcified foci, a few connective tissue fibres were seen to encircle incompletely some of the calcified single foci, external to which was found a wide (1-1.5 mm.), typical tuberculogenic granulation tissue, which macroscopically gave the impression of a definite capsule. This granulation tissue imperceptibly disappeared into the adjacent normal lymph node tissue.

The intermediate zone, mentioned above as situated between the capsule and the necrotic centre, was best seen in larger nodules and consisted of a loose granulation tissue with a circular arrangement of the fibres. Its width was variable, but in most cases it was about two or three times as wide as the corresponding true capsule. This granulation tissue was composed of a network of undifferentiated fibres with many fibroblasts, lymphocytes, macrophages and epithelioid cells. In some of the nodules, where the central necrotic mass was irregular rather than spherical, the granulation tissue filling the space between the capsule and the necrotic centre had a more irregular pattern, then strongly resembling tuberculogenic granulation tissue. The resemblance was emphasized by the presence in this intermediate zone of few to many multi-nucleated giant cells in addition to the epithelioid cells.

In lesions of pinpoint size, the intermediate zone mostly was absent or indistinct, so that the usually calcified centres were directly in contact with the fibrous capsule.

The central portion in the larger nodules was usually in an advanced stage of caseous necrosis. Usually several or many irregularly fused calcified foci were seen either centrally or in the periphery of the necrotic mass in dryish or gritty lesions, but calcium deposition was apparent also in the lesions with caseous or even soft, toothpaste-like content. In such lesions there was a uniform fine dispersion of the mineral salts all over the caseous material. In the firmer nodules, however, these salts were concentrated in the periphery of the necrotic centre in the form of a darker stained zone. In a number of lesions, smaller or larger calcified foci were seen scattered also in the intermediate or granulation tissue zone. Exceptionally, odd lesions were seen in sections in the form of non-caseous, conglomerate tubercle-like units composed of small tubercles with the characteristic cells of tuberculosis. These conglomerate units were as a rule encircled by a more or less definite fibrous capsule. *C. equi*, but not tubercle bacilli, were recovered from these lesions.

In a few nodules, where macroscopically a phenomenon of concentricity was seen, this was recognised also histologically, where the following zones could be distinguished, starting from without. Firstly, a distinct and usually dense fibrous capsule; next an intermediate zone of granulation tissue about twice as wide as the former. The intermediate zone could be divided again into an outer, darker stained zone with a predominance of lymphocytes, and an inner, less cellular and more faintly stained zone of a rather loose character, containing scattered epithelioid and odd giant cells. In the necrotic or caseous central mass again a separate layer or zone was formed by the deposition of calcium salts in greater concentration in the periphery. In some lesions this zone was formed by the presence of solid calcified foci in a continuous row on the periphery of the caseating mass. When such calcified foci were present also in the geometric centre, an additional zone was superimposed due to the less intensively stained portion of the homogeneous caseous material between the central and peripheral rows of calcified foci.

As a rule, the caseous mass was spherical or oval and distinct from the surrounding intermediate zone. In many sections the central portion was lost during preparation. In others, however, there was often a break between the intermediate zone and the caseous mass, indicating the line where the break in the continuity of the structures was likely to occur during enucleation of the material.

Outside the nodules, in the lymphoid tissue, some infiltration with eosinophiles was evident. This eosinophilia was especially marked in the sinuses of the lympho-reticular meshwork in the medullary zone (situated in the pig in the periphery of the lymph node), or even more prominent in the hilus portion of the lymph nodes. Eosinophiles were often present in such numbers that the sinuses in many places were packed with these cells.

Mottled Lymph Nodes.

On histological examination, proliferating and interlacing strands of the trabeculae were seen which were usually more prominent than in the normal portion of the node. The fibres of those strands had a somewhat swollen appearance, and in one node showed hyaline degeneration in a section of a strand. Between and branching from the main strands were finer strands of fibres. The space between the main strands was closely beset with many vacuoles ranging from a very small size up to 1 mm. in diameter. These vacuoles occurred in such numbers that when stained sections were held against a light, the affected area had a sieve-like appearance. In gelatin embedded blocks the cut surface revealed those holes as depressions of variable depth and size even before cutting the sections. The larger vacuoles occurred usually singly, but the medium and the small ones were mostly confluent or in chains, the separating septa or membranes being often broken, giving the area a close resemblance to fat tissue. However, no fat was found in the gelatin embedded or frozen sections when stained with Sudan. The large vacuoles occurred mainly in the primary nodules, occupying or replacing the germinal centres. The lymphocytes in the peripheral part of the primary nodules were usually

pushed towards one pole or compressed to a narrow strip against the fibrocytic enclosure of the primary nodules. Some of the large vacuoles had apparently collapsed, causing the outer lymphocytic rim to loosen from the encircling fibrocytic ring and to protrude tongue-like into the empty lumen of the vacuoles. As a whole, histologically this mottling had not the slightest resemblance to a tuberculous process.

All the mottled nodes examined showed a marked infiltration with eosinophiles, as described for the pseudo-tuberculous lymph nodes.

Normal Lymph Nodes.

Twenty of the 75 normal specimens were studied histologically. No detectable changes were observed except a marked infiltration with eosinophiles in all nodes, and small extravasations of red cells in the injected nodes. The infiltration with eosinophiles was about of equal degree, whether the nodes yielded *C. equi* or other organisms or were bacteriologically sterile.

DISCUSSION.

Incidence of Tuberculosis-like Lesions in Pigs.

The present study has shown that in Queensland in the submaxillary nodes of pigs lesions occur which macroscopically resemble tuberculosis, but are not caused by tubercle bacilli. These tuberculosis-like lesions have been observed by Plum (1940a) in Denmark in up to 1%, and by Rangsit (1940) in Switzerland in 2.4% of slaughtered pigs.

No data on the incidence of such lesions in Queensland have been published. On the limited observations in the Doboy bacon factory on the days when collecting the specimens, the incidence seemed to vary between 3·5% and 7% of total daily killings. In another Queensland bacon factory, however, according to local information, only about 0·5% or less were said to be affected. As the pigs were drawn from the same districts, it is possible that the difference was mainly due to local meat inspection conditions.

In South Australia, Collins (1939) reported apparently similar lesions. Of the 5,514 pigs slaughtered in a period during 1937, 11·1% showed lesions in the head lymph nodes. Most of the lesions were found to be tuberculous, but many were thought to be of parasitic origin, though no larvae were found.

Cause of the Lesions.

Meat inspectors in Queensland have for many years referred to these pseudo-tuberculous nodules as "worm" nodules, apparently on the assumption that they are caused by migrating larvae of the kidney-worm (*Stephanurus dentatus*), though no laboratory or other records providing a basis for this assumption can be found. Probably the publication by Ross and Kauzal (1932) of their results of the experimental infection of pigs with *Stephanurus* larvae, which by skin penetration caused caseous encapsulated nodules in the precrural lymph nodes, has contributed to some extent to this fairly widespread belief that lesions in the submaxillary nodes are of the same origin.

In this study certainly there was no evidence of parasitic invasion in the kidney region, nor were larvae encountered in the histological sections studied, whether normal or diseased.

As *C. equi* was recovered in more than 90% of these cases in Queensland, the evidence that this organism is a major contributing cause is strong. This is supported by the findings of McDonald (1942) and Woodroofe (1950) in South Australia, where *C. equi* was also isolated.

Role of Corynebacterium equi.

Ever since *C. equi* was first isolated in Scandinavia from tuberculosis-like lesions, there has been much discussion about its etiological role. It is evident that this organism has been isolated from affected submaxillary nodes of pigs with varying frequency in different countries (see Table 5).

Table 5.

RECOVERY OF *C. equi* AND/OR *M. tuberculosis* FROM DISEASED SUBMAXILLARY NODES.

Author.	Year.	Country.	Number of specimens examined.	Percentage of specimens.	
				C. equi.	*M. tuberculosis.*
Holth and Amundsen ..	1936	Norway	162	64·2*	63·5
Plum	1939	Denmark	1,280	26·0†	43·4
Plum	1940*b*	Denmark	2,028	35·4‡	0·2
Ottosen	1941	Denmark	150	73·3	..
Jepsen	1940	Denmark	400	44·2	45·0
Magnusson	1938	Sweden	146	15·0	58·2
Magnusson	1940	Sweden	235	30·2	97·4
Meyn and Müller ..	1940	Germany	100	29·0	6·0
Hemmert-Halswick and Pescatore	1948	Germany	48	8·3	81·1
Orlop	1951	Germany	34	11·7	55·8
Rangsit	1940	Switzerland ..	42	57·1	..
Verge and Senthille ..	1942*a*	France	16	25·0	..
Cotchin	1943	Great Britain ..	86	9·3	5·8
Pullin	1946	Canada	232	21·1	70·6
Feldman *et al.*	1940	U.S.A.	89	22·4	61·8
Woodroofe	1950	South Australia ..	28	53·5	..

* = Demonstrated in 38 out of 59 diseased specimens where no tubercle bacilli were recovered.

† = Results obtained by culturing on Löwenstein medium.

‡ = Results obtained by microscopic examination (no cultivation), but *C. equi* was frequently demonstrated even in up to 80 % of the microscopically negative specimens.

In general, tubercle bacilli have been found more frequently than *C. equi* in the diseased nodes. Inoculation or feeding trials in young pigs with pure cultures of *C. equi* by Karlson, Moses and Feldman (1940), Cotchin (1943), and Pullin (1946) failed to produce lesions, contrary to the observations of Bendixen and Jepsen (1938, 1940). Karlson, Moses and Feldman, and Cotchin, therefore concluded that *C. equi* has no etiological relationship whatsoever to

these lesions. Furthermore, Karlson and his associates were able to recover *C. equi* just as frequently from normal nodes as from diseased nodes. Cotchin, however, reported that only one of 50 normal lymph nodes harboured *C. equi*. McDonald (1942) and Woodroofe (1950) found *C. equi* but no tubercle bacilli in affected nodes, while Woodroofe (1950) isolated *C. equi* only once from 13 normal specimens.

In the present study the recovery of *C. equi* from tuberculosis-like lesions in Queensland was considerably higher than in South Australia, and even exceeded the highest figures from Scandinavia. Its recovery in 29·3% of the normal specimens is also considerably higher than in South Australia and compares with the findings in U.S.A.

In this study no tests for pathogenicity were done, but the high incidence of *C. equi* often (in 40%) in pure culture leads to the conclusion that it must be in some way responsible for these lesions. It is possible that the role of *C. equi* in tuberculosis-like lesions is somewhat overshadowed in other countries by the high incidence of tuberculosis, especially of the avian type, for when found in conjunction with *C. equi* the tubercle bacillus must be regarded as the organism of primary importance. In the absence of the tubercle bacillus, however, the presence of *C. equi* may be assumed to be of much more significance. In this study *C. equi* was so found in a high percentage of cases.

The failure of several workers to reproduce specific lesions by administering pure cultures of this organism by mouth or by inoculation is not sufficient to deny it pathogenic properties. Plum in a preliminary report (1939) stated that *C. equi* is only slightly pathogenic for pigs, but later (1940a) reported that specific lesions were more readily produced when it was administered in conjunction with influenza virus.

In trials by several workers, the test pigs were usually submitted to a single oral or parenteral exposure, except a feeding trial over six days by Cotchin (1943). It is possible that a single exposure can be readily overcome by the defence mechanism of the host. Even repeated administration, say for six days, would not entirely correspond to natural conditions, where infection may spread over a long period.

Mode of Infection.

The observation that several pigs from the same herd were affected simultaneously with the same type of lesions, from which *C. equi* was always recovered, suggests a common source of infection, probably soil. There is support for this in the fact that *C. equi* has been recovered as a common soil saprophyte. In Denmark, Bendixen and Jepsen (1940) isolated it from 46 out of 65 soil samples, and Ottosen (1945) recovered it from cow dung. In New South Wales apparently the same organism has been recovered by Jensen (1934) from garden and grass soil and from alluvial clay, so it is likely that this organism may be a soil saprophyte in Queensland also, though

this point has not been investigated so far. The fact that *C. equi* was recovered during this study from over 50 widely dispersed herds strongly suggests that it may be prevalent in the whole State, or even all over Australia.

It seems probable that due to their unclean feeding habits pigs ingest *C. equi* with contaminated soil. There is also evidence that *C. equi* may enter via the air passages, as Orlop (1951) in a few cases was able to isolate this organism from the lung lymph nodes.

That *C. equi* often does not produce visible lesions in the lymph nodes is probably correlated with their small numbers, with individual resistance of the host, or with some unknown factors. It is known that occasionally tubercle bacilli may be isolated from lymph nodes that show no macroscopic or microscopic lesions.

Role of Other Bacteria.

The frequent recovery of bacteria other than *C. equi* in these tuberculosis-like lesions has been referred to by Feldman, Moses and Karlson (1940), who suggested care in ascribing the lesions to any particular organism. Similarly, Ginsberg and Fitzpatrick (1950) were in doubt as to which of the bacterial types they isolated—corynebacteria (*C. pyogenes* mainly), *Escherichia coli,* haemolytic streptococci, bipolars or acid-alcohol-fasts—is the primary invader.

In this study, the variable frequency of the miscellaneous bacteria suggests that they play little part in the genesis of the lesions, because they were met with far less frequently than *C. equi,* and then usually in association either with the latter or with tubercle bacilli, or with both. Haemolytic streptococci and micrococci were the most common organisms, followed by staphylococci, various saprophytic acid-fasts, gram-negative organisms and lastly *Proteus.* Only in the "mottled" group were these organisms, especially streptococci, encountered by themselves, which suggests a possible etiological role. Furthermore, Holz (1952) has stressed the harmful effects of streptococcal infection of the pig in Germany. In the lymph nodes a marked infiltration with eosinophiles was reported. This last phenomenon, however, was so common in this study, and was pronounced not only in the diseased but also in apparently normal lymph nodes, many of which were completely sterile or often yielded bacteria other than streptococci, that one is compelled to regard eosinophilia as a normal feature of the pig's lymph node rather than one due to streptococcal or any specific infection.

That streptococci were in any way responsible for mottling or any other lesions is contradicted by the equally high recovery of streptococci, as a rule in combination with other bacteria, from normal nodes.

The possibility of other bacteria playing a role was eliminated by the fact that in a number of cases *C. equi* was the only organism recovered from the content of the pseudo-tuberculous nodules, whereas the surrounding lymphoid tissue yielded abundant other bacteria, but few or no *C. equi.*

Whether the saprophytic mycobacteria recovered in this study have any particular role is difficult to ascertain, as they were never encountered alone but always in association with corynebacteria.

The possibility of Brucella infection being the cause of these lesions was ruled out, as no specific agglutinins were demonstrated in any of the guinea pigs inoculated with the material from the diseased nodes.

Macroscopic Differentiation of Pseudo-tuberculous and Tuberculous Lesions.

The important question from the meat inspection point of view is whether tuberculosis-like lesions due to *C. equi* can be distinguished from tuberculosis with reasonable certainty without laboratory aid. This is already affirmatively answered by the fact that the initial classification by the meat inspectors, when collecting the specimens for this study, was found later to be accurate. Nevertheless, it should be noted here that different opinions, often controversial, have been expressed in the literature on this question.

Holth and Amundsen (1936), Jespersen (1938) and Bendixen and Jepsen (1938) stated that the lesions caused by *C. equi* can be readily distinguished from tuberculosis. Corynebacterial lesions in general were described as consisting of discrete nodules, irregularly spherical and containing a faint yellowish necrotic mass surrounded by a distinct capsule. The nodules were readily enucleated from the capsule, leaving a smooth, well-defined depression. Keller (1951) found nodules with similar characteristics in wild swine in Germany. The porridge-like or laminated content was readily expressed, because there was no such close union with the surrounding tissues as occurs in tuberculous foci. In pseudo-tuberculosis there was no calcification such as occurs in tuberculosis, and each small abscess was clearly separated from the adjacent ones by a distinct capsule. Tuberculous foci, on the contrary, never show such delimitation, and only very infrequently a smooth capsule is present.

Rangsit (1940) also was able to tell macroscopically which type of lesion was present. The tuberculous submaxillary nodes were usually enlarged and hardened, but this never occurred in corynebacterial infection. However, in some cases that yielded tubercle bacilli, the nodes were normal in sizes and consistency. On the cut surface they showed some few nodules the size of a pinhead, so doubt about their true character arose occasionally, but these lesions were only with difficulty shelled out, had no capsule and were rather irregular in shape. In the true corynebacterial lesions, it was only in the smaller nodules of about 1 mm. in size that a capsule was not detected, but the lesions were loose in the tissue.

Though Plum (1946), Feldman, Moses and Karlson (1940), Hemmert-Halswick and Pescatore (1948) and Orlop (1951) were unable to distinguish between the different lesions, the present study has brought evidence that corynebacterial lesions can be differentiated from tuberculosis. The tuberculous nodes in general were characterized by a marked swelling and fibrosis, which was not seen in pseudo-tuberculous nodes. Only in a few cases was there no

enlargement, and the tissue then contained only small discrete nodules without a capsule. These nodules superficially resembled corynebacterial lesions, but on closer inspection showed those features reported by Rangsit (1940) in doubtful cases—namely, instead of being spherical the lesions were very irregular, no capsule was present, and it was difficult to enucleate them from the lymph node tissue. These features distinguished them from typical corynebacterial lesions.

Encapsulation was the rule for the larger corynebacterial lesions, but in the smaller nodules, less than 2 mm. across, it can be frequently missed by the naked eye. In such cases one has to rely on the appearance of the larger nodules when they are present. Sometimes a capsule can be missed macroscopically also in the larger nodules, but it is seen on microscopic examination. When the inspector in in doubt, it is wise to classify them as tuberculosis.

However, it seems that capsule formation is not restricted to the corynebacterial lesions only. Pallaske (1931) referred to a capsule around the nodular form of lymph node tuberculosis in pigs caused by the bovine type, and Feldman (1949, p. 342) in a microphotograph showed a distinct capsule around a tuberculous nodule in the spleen of a pig caused by the avian type. A well-defined capsule is described also in tuberculous foci in man (Anderson, 1948, p. 264). In this study, the tuberculous lesions, which were apparently due to the bovine type of infection (avian type of tubercle bacilli have so far not been recorded in Queensland), never showed such a distinct connective tissue capsule as was seen in the corynebacterial lesions.

Some standard textbooks of meat inspection (e.g., Thornton, 1949, p. 272) describe tuberculous lesions as readily enucleated nodules. Pallaske's (1931) paper on tuberculous nodules in pig's lymph nodes stated that this is due to a fermentative process in the regressive stage of the bovine type of infection. In this study, however, ready enucleation was not observed in tuberculous foci. As the nodules mentioned by Pallaske were encapsulated, there is a possibility that he may have been dealing in some cases with lesions of corynebacterial origin, the occurrence of which had not been reported at that time.

The concentric arrangement of the content, which Pallaske has described for the tuberculous lesions as due to an intermittent deposition of calcium salts, and is also mentioned as occurring in man (Anderson, 1948, p. 264), seems to occur in both types of lesions, as this lamination has been reported by Keller (1951) and observed also in a few cases in this study in corynebacterial lesions.

Calcification of the lesions was stated by Keller to be characteristic of tuberculous nodules only, but this was not confirmed by this study. In fact, deposition of calcium salts was evident histologically in all cases, regardless of the consistency of the nodules. It was present even in the softest material, and was then uniformly dispersed. In the firmer or gritty nodules, solid calcified foci were scattered in the caseous material either on the periphery or in the centre of the necrotic mass.

Ginsberg and Fitzpatrick (1950) considered that one could determine the type of infection (e.g., corynebacterial, streptococcal, &c.) by the consistency or by the colour of the lesions, but this was not supported in this study. It may be true that a greenish discolouration indicates *C. pyogenes,* which they recovered in about one-third of their specimens. In this study, no *C. pyogenes* was isolated, though in several specimens a light greenish discolouration of the nodules was apparent. There was no evidence to suggest that the consistency of the lesions was in any way associated with any particular type. *C. equi* was recovered from lesions of all consistencies—soft, cheesy, dry and calcareous. The same applied to the colour of the lesions, three-fifths of which were cream-coloured, one-fifth whitish and one-fifth slightly yellowish, but all yielded *C. equi.* Similarly, the incidence of other miscellaneous bacteria was not in any way correlated with any particular colour of the lesions.

Microscopic Differentiation.

It was found difficult to differentiate corynebacterial infection from tuberculosis by histological study, mainly because the tissue reaction in both infections is similar. A point of difference is that a dense fibrous capsule seems to be a more or less constant feature in corynebacterial lesions. It was frequently found that a single or conglomerate epithelioid-cell-tubercle formation, which is said to be typical of swine tuberculosis (Nieberle, 1931), was met with also in corynebacterial infection where no caseation had occurred, thus suggesting tuberculosis. However, the negative bacteriological findings did not support this. Even if it is assumed that the statement by Fresen (1950) that a diagnosis of tuberculosis in man can be based solely on the presence of typical miliary tubercles is correct, the evidence supplied here does not support the view that this holds for tuberculosis in pigs. In corynebacterial lesions the same cell elements that characterize tuberculosis—namely, giant and epithelioid cells, especially the latter—are encountered so frequently that their absence is rather an exception. As for the giant cells, they were absent in approximately 50% of the corynebacterial cases, nor are they invariably present in lymph node tuberculosis.

Eosinophilia.

The eosinophilic infiltration of the lymph nodes, which was common in corynebacterial infections and to a lesser degree in the tuberculous nodes, is of doubtful significance. An eosinophilia has been described as a feature of certain chronic conditions in man (Vines, 1949, pp. 562, 593). It has been reported also in the avian (Pallaske, 1931) and bovine (Hemmert-Halswick and Henninger, 1938) type of chronic lymph node tuberculosis of pigs, but was not seen by Feldman (1938, p. 334), who found only very few or no eosinophiles. In fact, an eosinophilia of the lymph nodes was found in this study to be common in both normal and diseased nodes, so it is probably a regular feature of the porcine lymph nodes.

"Mottling."

The whitish net-like mottling described above has no relation to a tuberculous infection, though macroscopically the lesions resembled to some extent a tuberculous streaky necrosis mentioned by Emanuiloff (1939), who reported the same condition in pigs in Bulgaria and other European countries. According to him it occurred in young pigs (4 months) in the lateral retropharyngeal nodes only, but with increasing age (8-12 months) some other lymph nodes, such as the parotid and cervicals, but never the submaxillary nodes, may become involved.

Contrary to his observation, this net-like mottling was found often in the submaxillary nodes of pigs aged from 5 months to 9 months. Whether additional nodes were involved also cannot be said, as they were not examined.

Though various organisms were recovered from these mottled nodes, no particular bacterial type could be selected as the causal infective agent. Proliferation of the trabeculae with collagenic metaplasia of the fibres suggests a healing process in the lymph nodes following a previous inflammation. Fat resorption, as reported by Emanuiloff (1939) in affected lymph nodes, could not be detected in the submaxillary nodes. Though in sections a large number of vacuoles of variable size and often confluent were seen in the affected areas, the presence of fat in the vacuoles was not revealed by appropriate staining methods. It is possible that these vacuoles were originally extended sinuses which contained an inflammatory fluid which may have escaped during the preparation of the sections.

Trabeculae were more numerous in the affected areas than in the normal portions of the sections, but the histology still left doubt whether these strands alone were responsible for the net-like pattern. Macroscopically the interwoven lines appeared to be more numerous and closer interlaced than was revealed by microscopic examination. It is possible that the rows of vacuoles gave a false impression of strands.

Acid-fastness of C. equi.

This study has largely confirmed the reports by the Scandinavian and several other workers (Hemmert-Halswick and Pescatore, 1948; Rangsit, 1940; Orlop, 1951, and others) of the occurrence of acid-fast forms in *C. equi*, which, however, was denied by Karlson, Moses and Feldman (1940), Cotchin (1943), and Bruner and Edwards (1941), or observed only in old cultures by Verge and Senthille (1942b). The presence of distinct reddish or bright-pinkish-stained ovals or even bacillary forms of *C. equi*, which had resisted the action of a strong decolorizer such as 25% HCl, cannot be explained otherwise than by conceding acid-fast properties to a certain percentage of these organisms. There is an indication also that the incidence of acid-fastness is somewhat correlated with the composition or nature of the culture medium.

ACKNOWLEDGMENTS.

The helpful advice of Mr. G. C. Simmons, B.Sc., during the course of this investigation, and the co-operation of the meat inspectors, particularly Mr. R. J. O'Sullivan of Doboy Bacon Factory, in securing and classifying the material for the study, is acknowledged.

Thanks are due also to Prof. J. Francis, of the Veterinary School, University of Queensland, for identifying the streptococcal strains; to Dr. J. Legg, D.V.Sc., and to Mr. A. K. Sutherland, B.V.Sc., M.S., who gave valuable suggestions and also revised the manuscript; and to Mr. A. V. Robinson for the microphotographs.

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FURTHER STUDIES OF FLUOROSIS IN MERINO SHEEP.

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SUMMARY.

Further studies were made on six sheep which had been continuously exposed to fluorided water from 3 months to 27 months of age. They were maintained for a period of 30 months during which they had access to grazing and grassy lucerne hay and were protected from fluorine in the drinking water.

Observations were made on the permanent incisor teeth. Autopsies were carried out at the end of the 30 months on fluorine-free water. X-ray photographs were taken and bones, teeth and some organs were analysed for fluorine. Comparison of these data with those previously reported for similar sheep immediately after the period of exposure to fluorine supports the following conclusions:—

(1.) The effects of fluorosis on the incisor teeth are permanent. The brittleness of affected teeth and the lines of weakness at bands of pitting result in increased rather than decreased damage. Teeth which erupt after 12 months' protection contain less fluorine and are less severely affected, but positional defects at eruption still result in a badly deformed incisor arch.

(2.) Bone defects such as rarification and shortening of the horizontal ramus of the mandible, which were apparent at the end of the period of exposure to fluorine, are not permanent.

INTRODUCTION.

Fractures and excessive wear of the teeth are constant features of fluorotic Merino sheep that exist solely by grazing natural pasture in the field. This is not true of experimentally induced fluorosis in penned sheep. It has been assumed that the lesions are accentuated in the field by the fibrous food which for several months of each year represents the bulk of the grazing.

Experimental sheep with a known history of fluorosis were available to test the hypothesis. The work was superimposed on a study involving the recuperation of young fluorotic sheep kept on fluorine-free water and the results are now recorded together.

The objects of the investigation were:—

(1) To study the effects of grazing or simulated grazing on the teeth of sheep already damaged by fluorosis.

(2) To obtain information on fluorine storage in bones and teeth after 24 months' exposure to and 30 months' protection from water containing excessive fluorine.

(3) To make further observations on the bone rarification recorded in previous studies.

EXPERIMENTAL.

The six experimental sheep were bred in south-western Queensland from ewes which had not been exposed to water containing more than 0·5 p.p.m. fluorine. As 3-4-month-old lambs they were transferred to the Animal Health Station, Yeerongpilly, and were used immediately for two years in the experiment previously reported (Harvey, 1952). During that period they were housed in pens and fed cereal chaff plus milled supplements. The drinking water for some contained 10 p.p.m.F and for others 5 p.p.m.F.

For the following 30 months they had access to grazing, mainly a couch (*Cynodon dactylon*) and paspalum (*Paspalum dilatatum*) pasture. Grassy lucerne hay was fed in bales. The sole drinking supply was city water, which did not contain more than 0·2 p.p.m.F.

Observations were made on the incisor teeth at monthly intervals. Photographs of the incisor teeth were taken at 27 months, 51 months and 57 months of age. After autopsy at 57 months of age, X-ray studies were made on femur, tibia and mandible. Fluorine analyses were done on muscle, kidney, femur, tibia, metatarsus, mandible and incisor, molar and premolar teeth.

RESULTS.

Table 1 records the observations on the incisor teeth of the six experimental animals. Figs. 1–6 show the incisor teeth after 24 months' exposure to fluorided water and after a further 24 months and 30 months, during which they were protected from fluorided water.

Table 2 records the fluorine content of muscle, kidney, bones and teeth from the six sheep. Examination of the analytical data shows the following features:—

(1) The concentration of fluorine in both kidney and muscle is similar to that recorded at the end of 24 months' continuous exposure to fluorine in the drinking water.

(2) In all six sheep, there is a marked reduction in the fluorine concentration in femur and mandible when compared with levels recorded in sheep of the same group slaughtered 30 months previously, at the conclusion of the period of exposure to water containing fluorine. This reduction in fluorine concentration is most marked in the mandible. The concentration of fluorine also shows a tendency to fall in the tibia, whereas fluorine levels have remained relatively constant in the metatarsus.

(3) The fluorine concentration in the incisor teeth does not differ markedly from levels recorded immediately after the exposure period. The levels of fluorine in the 4th pair of incisors are much lower than those found in the 2nd and 3rd pairs. This difference is less marked in sheep F, but the explanation lies in the fact that the 4th pair of incisors in this sheep had erupted

within four months of transfer to fluorine-free water. For sheep A, B, C, D, and E, eruption of the 4th pair of incisors did not occur for 12 months or longer after transfer to fluorine-free water.

(4) The fluorine levels in premolar and molar teeth tend to be higher than the levels recorded previously in similar sheep slaughtered immediately after exposure to fluorine.

X-ray photographs of the femur, tibia and mandible are shown in Figs. 7–18. In none of the sheep is the bone rarification noted previously at 27 months of age apparent at 57 months of age. There has been marked lengthening of the horizontal ramus of the mandible, and the roots of the molar and premolar teeth no longer extend into the compact substance. There is evidence of uneven wear on both molar and premolar teeth in some sheep.

DISCUSSION.

All the incisor teeth remaining at the conclusion of this investigation show the characteristic lesions associated with fluorosis. These lesions are emphasised by staining due to degradation products of substances present in the diet during the grazing period only. In each of these six sheep the damage from fluorosis has been most marked in the 3rd pair of incisors. The damage to the 4th pair is less severe, but in all sheep one or both of these incisors have erupted at right-angles to the normal plane. A badly deformed incisor arch has resulted in all sheep. The shedding of the 1st pair and in some cases the 2nd and 3rd pair of incisors probably arose from the 24-month period from 3 months to 27 months of age when these sheep were maintained entirely on chaffed or milled feed. In areas of endemic fluorosis in Queensland, although excessive wear and breaking of incisors at lines of pitting have been characteristic, the shedding of incisors has not been noted.

Unevenness of the cutting surfaces of the molars is noticeable in the X-ray photographs from some of these sheep. It is less severe than that noted in specimens from endemic areas. The explanation probably lies in diet. First, these sheep were maintained from the age of 3 months to 27 months on chaffed feed; and secondly, from the age of 27 months to 57 months they were fed pasture and lucerne hay—this would involve less wear on the molar teeth than the harsh stubble-like pasture that is often the sole source of feed for many months of each year in parts of Queensland where fluorosis is endemic.

X-ray examination shows that the bone rarification, noticeable at the conclusion of the 24-month exposure period, is not apparent at the conclusion of the 30-month protection period. For each of these six sheep there has been a considerable thickening of the compact substance of the femur and tibia, and more particularly of the mandible. There has also been a marked lengthening of the horizontal ramus of the mandible. Overcrowding of the molars, which was apparent earlier, and which resulted in irregular cutting surfaces, is no longer noticeable.

Table 1.

COMMENTS ON INCISOR TEETH.

Sheep.	History from 3 to 27 months of age.	Comments on Incisor Teeth.			
		At transfer to grazing. Age 27 months.	After 12 months' grazing. Age 39 months.	After 24 months' grazing. Age 51 months.	At slaughter after 30 months' grazing. Age 57 months.
A	Group 2A. Ration : oaten chaff + bonemeal. Water : 5 p.p.m.F.	2nd pair in wear ; all teeth show erosion, horizontal chalky bands and chipping of cutting edges ; a badly deformed incisor arch.	3rd pair almost in wear, erupted at right angles, chalky and show erosion emphasised by considerable staining.	1st pair, left only has been shed ; 2nd pair show increased staining at the base ; 3rd pair markedly eroded and stained.	1st pair, both shed ; 2nd pair as previously described ; 3rd pair as previously described ; 4th pair almost in wear, erupted at right angles, stained but not markedly eroded.
B	Group 2B. Ration : oaten chaff + bonemeal. Water : 10 p.p.m.F.	2nd pair in wear ; all incisors show horizontal chalky bands, erosion, chalky areas and slight chipping of cutting edges.	3rd pair in wear, markedly eroded and stained over whole surface ; some deep bands of pitting towards the gum margin ; broken cutting edge.	1st pair shed ; 2nd pair show increased staining ; 3rd pair as previously described ; 4th pair erupting.	2nd pair as previously described ; 3rd pair show increased damage to cutting edge ; 4th pair erupted at right angles, less chalky than other incisors but eroded and stained.
C	Group 4A. Ration : oaten chaff. Water : 5 p.p.m.F + calcium sulphate.	3rd pair erupting ; all incisors show marked horizontal striations, erosion and some chipping of cutting edges.	3rd pair in wear, markedly eroded and stained with deep bands of pitting.	1st pair, right only has been shed ; 2nd pair show chalky striations over the whole surface and bands of pitting emphasised ; 3rd pair markedly eroded and pitted and the right one at right angles.	1st pair, both have now been shed ; 2nd pair right only has been shed ; 3rd pair, the left one is now badly worn ; 4th pair at right angles, eroded and stained.

Table 1.—continued.

COMMENTS ON INCISOR TEETH—*continued.*

Sheep.	History from 3 to 27 months of age.	Comments on Incisor Teeth.			
		At transfer to grazing. Age 27 months.	After 12 months' grazing. Age 39 months.	After 24 months' grazing. Age 51 months.	At slaughter after 30 months' grazing. Age 57 months.
D	Group 4B. Ration : oaten chaff. Water : 5–7 p.p.m.F + calcium sulphate.	2nd pair in wear ; all incisors are elongated, paper white with some chalky areas ; crowding has produced an irregular incisor arch.	3rd pair erupting.	1st pair have been shed ; 2nd pair show bands of pitting emphasised by staining ; 3rd pair deeply eroded with staining over the whole surface ; 4th pair in wear, erupted at right angles, eroded and stained.	2nd pair as previously described ; 3rd pair as previously described ; 4th pair as previously described.
E	Group 6A. Ration : oaten chaff + peanut meal + limestone. Water : 5 p.p.m.F.	2nd pair in wear ; all incisors show marked horizontal striations; 1st pair show some deep pits near the cutting edge ; 2nd pair more chalky.	3rd pair erupting.	1st pair have been shed ; 2nd pair show increased staining ; 3rd pair splayed, markedly eroded and stained ; 4th pair, left only erupted at right angles.	2nd pair, staining emphasises band of pitting at base ; 3rd pair, as previously described ; 4th pair, left only erupted at right angles.
F	Group 6B. Ration : oaten chaff + peanut meal + limestone. Water : 10 p.p.m.F.	3rd pair erupting ; 2nd pair chalky and heavily striated ; 1st pair show marked surface erosion.	3rd pair in wear, markedly eroded and striated with chalky bands ; 4th pair erupted at right angles, heavily eroded and stained.	1st pair have been shed ; 2nd pair have been shed ; 3rd pair, right only has been shed, left only has been heavily eroded with bands of pitting ; 4th pair as previously described.	3rd pair, left only as previously described, but shows a broken cutting edge ; 4th pair chalky, eroded with some deep pitting.

Table 2.

FLUORINE CONTENT OF MUSCLE, KIDNEY, FAT-FREE BONES AND TEETH.

(p.p.m.F on dry-matter basis).

Tissue.	Group 2A.*		Group 2B.†		Group 4A.*		Group 4B.‡		Group 6A.*		Group 6B.†	
	Group Average at 27 months.	Sheep A at 57 months.	Group Average at 27 months.	Sheep B at 57 months.	Group Average at 27 months.	Sheep C at 57 months.	Group Average at 27 months.	Sheep D at 57 months.	Group Average at 27 months.	Sheep E at 57 months.	Group Average at 27 months.	Sheep F at 57 months.
Gastrocnemius ..	2·0	1·5	1·0	1·5	3·0	1·5	2·0	2·5	3·0	1·0	1·6	3·0
Kidney ..	8·8	20·0	14·6	20·0	9·2	28·0	16·8	52·0	26·2	16·0	43·4	35·0
Femur ..	1,300	1,320	2,600	1,800	1,600	840	1,600	800	1,030	1,080	1,750	1,240
Tibia	850	1,040	1,700	1,360	1,275	880	850	880	550	1,040	1,450	1,200
Metatarsus ..	725	1,040	1,600	1,520	1,100	880	825	800	525	800	1,125	1,040
1st Incisors ..	.....	Shed	.....	Shed	.....	Shed	....	Shed	....	Shed	....	Shed
2nd Incisors ..	840	960	1,600	2,560	1,600	1,000	1,170	960	740	900	....	Shed
3rd Incisors ..	1,520	800	1,540	1,600	1,540	1,370	1,540	1,000	810	1,200	1,760	1,500
4th Incisors ..	....	470	....	550	....	725	....	600	....	600	....	1,270
3rd Premolar ..	960	1,360	1,520	2,400	1,300	1,440	1,240	1,040	690	1,120	1,460	1,526
2nd Molar ..	780	880	960	1,520	920	1,200	920	1,120	400	1,120	1,160	1,120
3rd Molar ..	1,120	1,280	1,580	2,080	1,210	1,360	1,230	1,040	760	1,200	1,130	1,440
Mandible ..	1,960	960	2,000	1,200	2,480	800	1,800	650	1,200	960	2,480	1,200

* On 5 p.p.m.F in drinking water from 3 months to 27 months of age.

† On 10 p.p.m.F in drinking water from 3 months to 27 months of age.

‡ On 5-7 p.p.m.F in drinking water from 3 months to 27 months of age.

It was stated in discussing the earlier studies (Harvey, 1952) that the bone rarification noted after 24 months' exposure to fluorine may not have been entirely due to the fluorided water, and that other factors which may have contributed were the diet of chaffed or milled food and the confinement in pens. Further support to these conclusions is given by recent studies (unpublished data) on grazing sheep, in which bone rarification was not discernible at 33 months of age in a group continuously exposed to 10 p.p.m.F in the drinking water from the age of 5 months. From the present investigation it is apparent that the bone defects in these sheep are not permanent and have been largely overcome during the 30 months in which they had access to grazing or simulated grazing plus fluorine-free water. This is not true of the damage sustained by the incisor teeth. The dental lesions are permanent and the effects of erosion and wear are enhanced with age even though fluorine was excluded from the drinking water.

The fluorine concentration in the bones examined is in agreement with the X-ray studies. Comparison with fluorine levels found at autopsy after 24 months' exposure to fluorided water shows a marked reduction in the fluorine content, particularly in the mandible. This is in keeping with the marked increase in dense bone deposition during the 30 months when the animals were not exposed to fluorine.

The fluorine concentration in incisor teeth compares with levels recorded in similar sheep immediately after the exposure period. There has been a marked reduction in the fluorine content of the 4th pair of incisors when compared with that of the 3rd pair. This is particularly noticeable when the 4th pair did not erupt during the 12 months succeeding the exposure period. This is in agreement with the less marked lesions of fluorosis noted in this pair of incisors.

The fluorine levels in molar and premolar teeth tend to be higher than levels recorded immediately after the exposure period, when the eruption of the 3rd molars was in progress. This could be accounted for by wear on the exposed surface of the crown. The concentration of fluorine is much greater in dentine than in enamel, so any loss of enamel through wear on the masticatory surface of the tooth would result in an increase in the fluorine concentration in teeth of fluorotic sheep.

Consideration of the findings recorded in this study in relation to field practice stresses the importance of protecting sheep from fluorine during the susceptible age period when permanent teeth are being laid down. In marked distinction to bones, the effects of fluorosis on the incisor teeth are permanent. In fluorotic sheep incisor teeth erupting some 12 months after protection from fluorided water still show the pronounced lesions as well as positional defects.

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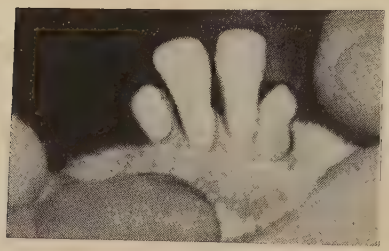

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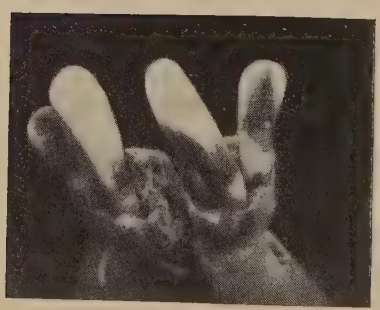
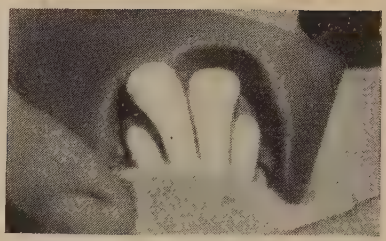
b



c

Fig. 1.

Incisors of Sheep A at (*a*) 27, (*b*) 51 and (*c*) 57 Months of Age.



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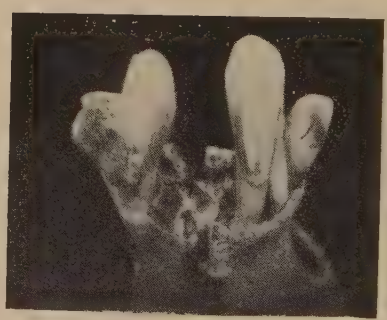

c

Fig. 2.

Incisors of Sheep B at (*a*) 27, (*b*) 51 and (*c*) 57 Months of Age.

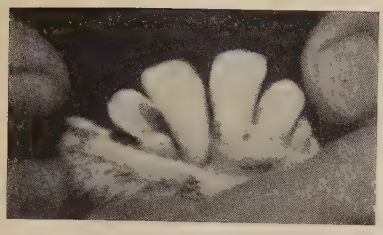

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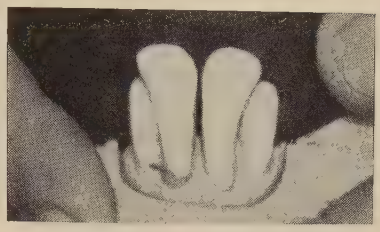
Fig. 3.

Incisors of Sheep C at (*a*) 27, (*b*) 51 and (*c*) 57 Months of Age.



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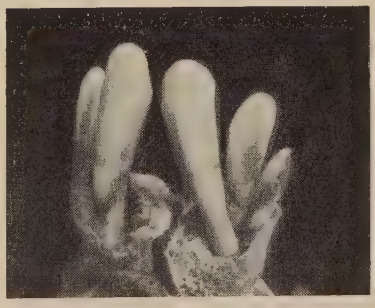


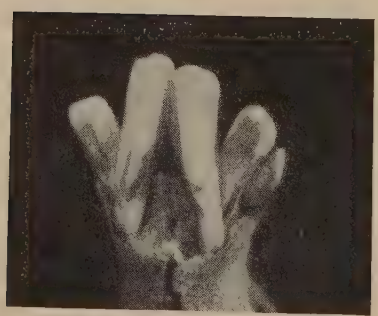
c

Fig. 4.

Incisors of Sheep D at (*a*) 27, (*b*) 51 and (*c*) 57 Months of Age.

a

b



c

Fig. 5.

Incisors of Sheep E at (*a*) 27, (*b*) 51 and (*c*) 57 Months of Age.

a

b

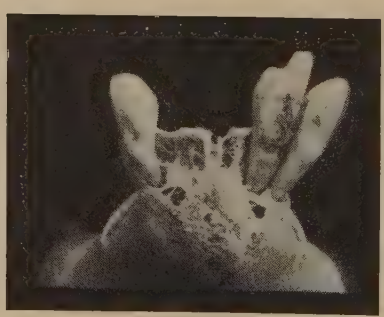


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Fig. 6.

Incisors of Sheep F at (*a*) 27, (*b*) 51 and (*c*) 57 Months of Age.

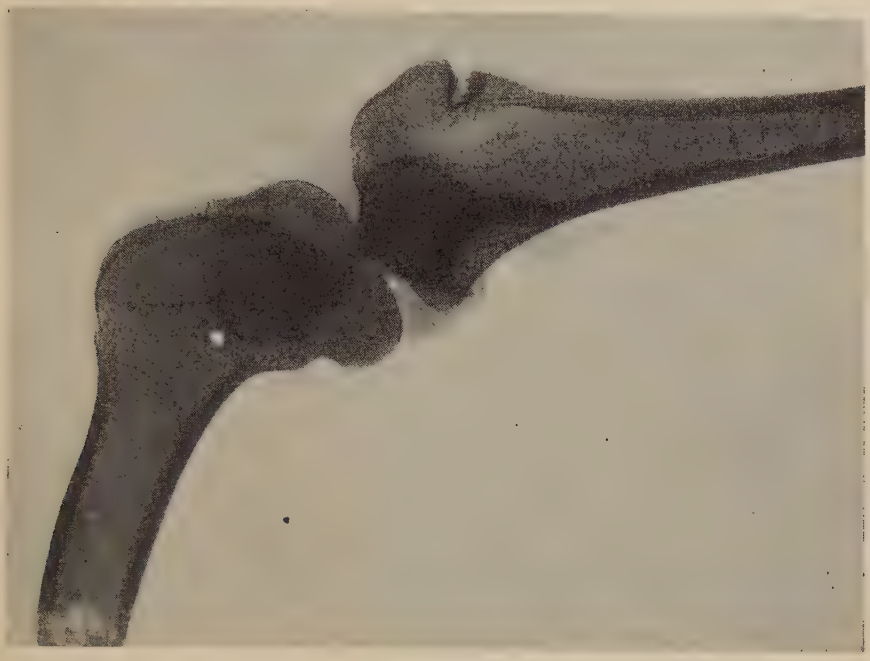

Fig. 7.
Femur and Tibia of Sheep A at 57 Months of Age.

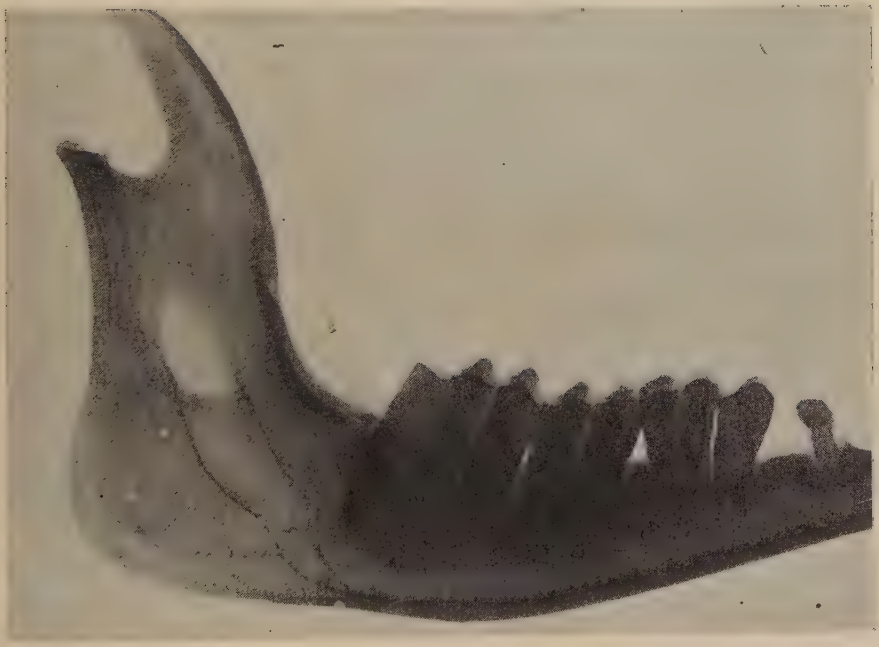

Fig. 8.
Mandible of Sheep A at 57 Months of Age.

Fig. 9.
Femur and Tibia of Sheep B at 57 Months of Age.

Fig. 10.
Mandible of Sheep B at 57 Months of Age.

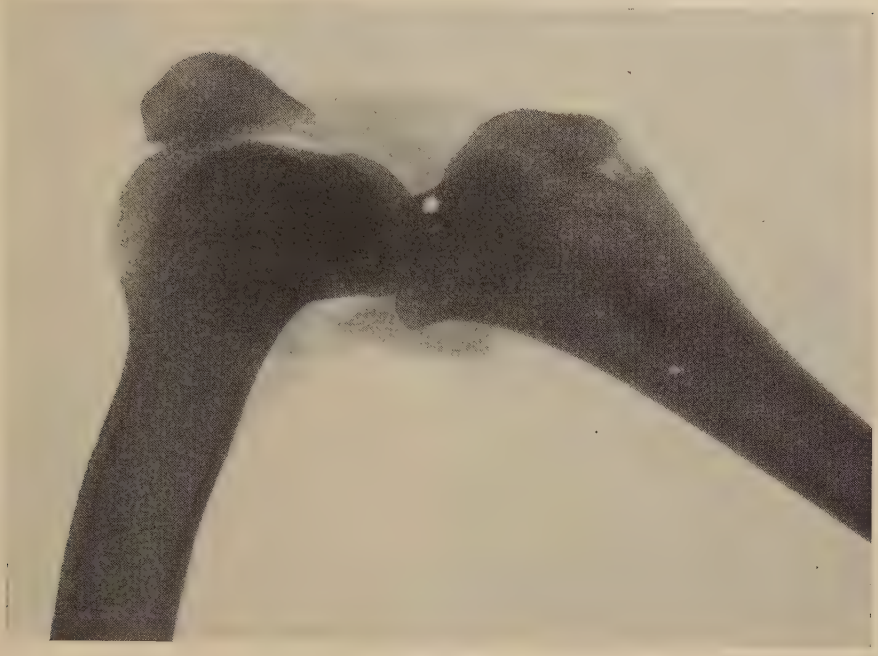

Fig. 11.
Femur and Tibia of Sheep C at 57 Months of Age.

Fig. 12.
Mandible of Sheep C at 57 Months of Age.

Fig. 13.
Femur and Tibia of Sheep D at 57 Months of age.

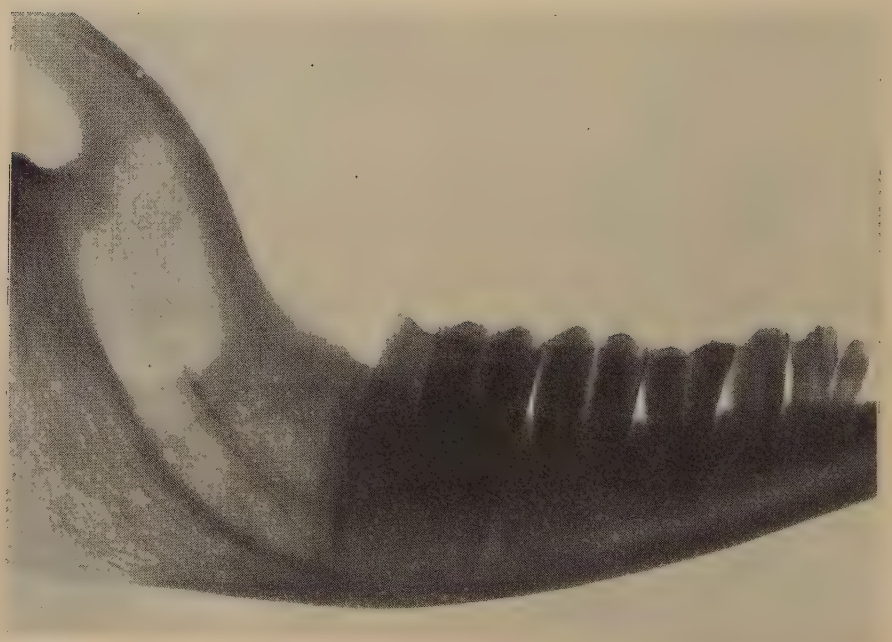

Fig. 14.
Mandible of Sheep D at 57 Months of Age.

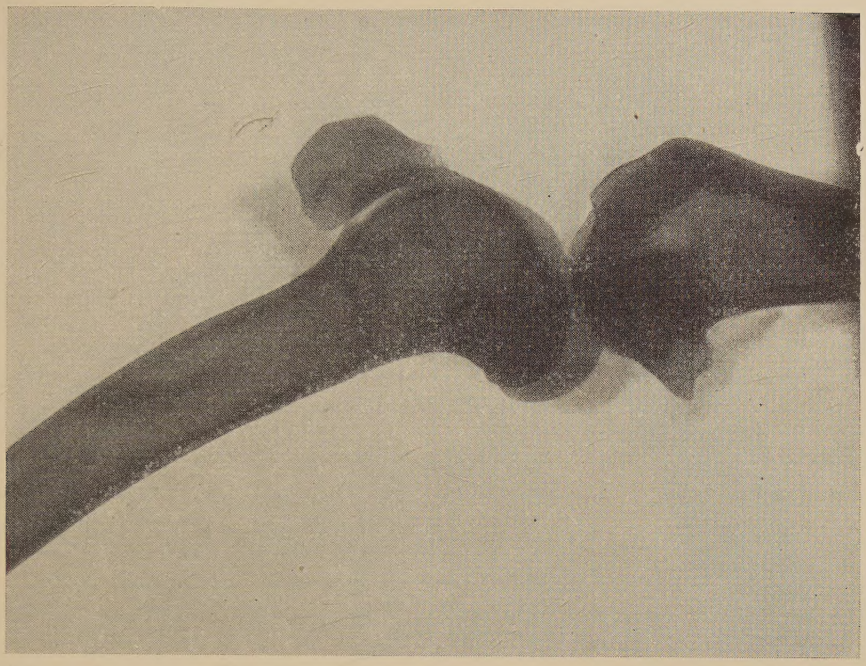

Fig. 15.
Femur and Tibia of Sheep E at 57 Months of Age.

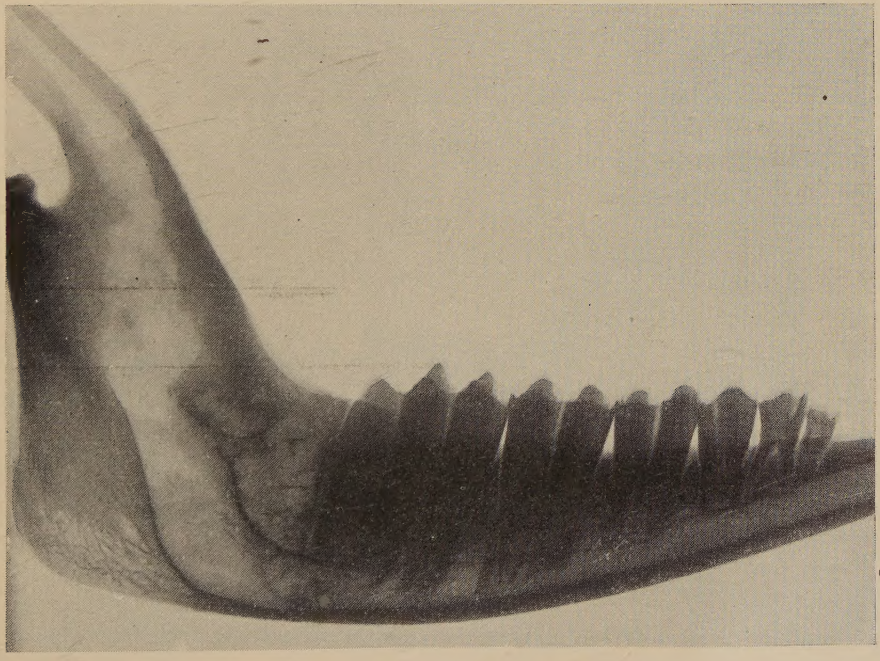

Fig. 16.
Mandible of Sheep E at 57 Months of Age.

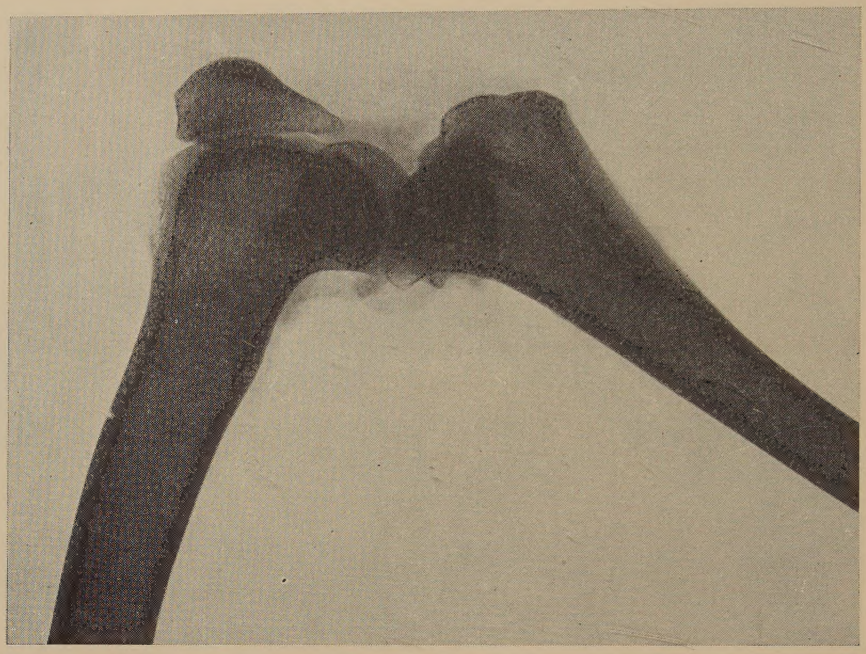

Fig. 17.
Femur and Tibia of Sheep F at 57 Months of Age.

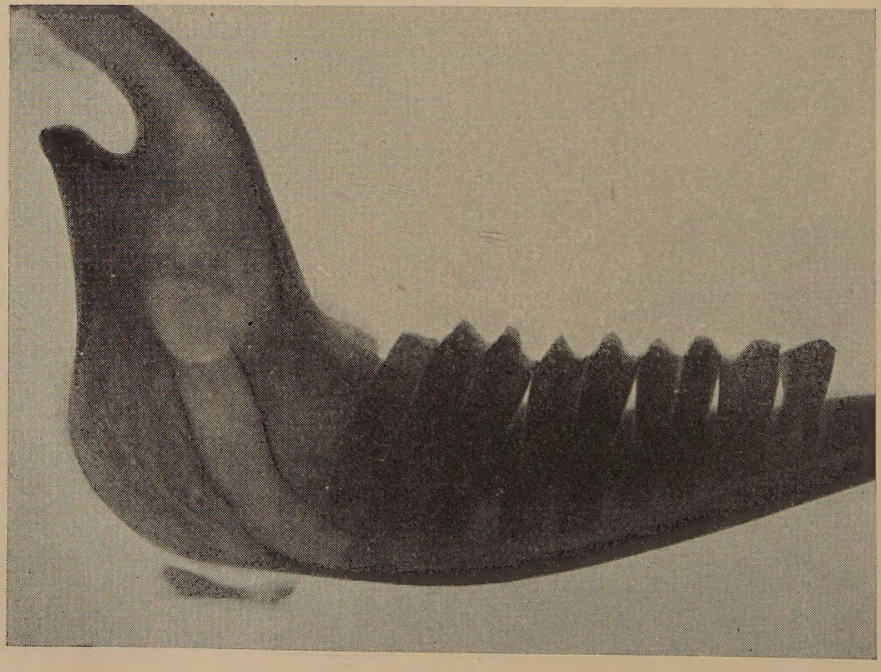

Fig. 18.
Mandible of Sheep F at 57 Months of Age.

TECHNICAL NOTES.

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Mould Growth on Sorghum Seed.

Many seed samples harvested from the 1952-53 sorghum crop in southern Queensland developed considerable mould growth during germination tests. This seriously hampered the work of the Seed Testing Laboratory.

The following different fungi were isolated from the seed samples:— *Alternaria* spp., *Aspergillus* sp., *Rhizopus* sp., *Heterosporium* sp., *Helminthosporium* sp., and *Fusarium* sp.

Three species of *Alternaria* predominated, and surface sterilization with 1:1000 mercuric chloride for 20 minutes, followed by washing, failed to eliminate their growth.

A series of tests comparing the efficiency of various fungicidal dusts in controlling mould growth were conducted, with the following results.

Treatment.	Laboratory Tests in Germination Trays.		Percentage Germination in Pots of Sterile Soil.
	Percentage Germination.	Percentage Mould.	
A : Copper carbonate			
1 oz. per bushel	70	26	61·2
2 oz. per bushel	67	27	61·2
4 oz. per bushel	73	28·5	53·2
B : Thiram (50%)			
1 oz. per bushel	70·5	0	69·2
2 oz. per bushel	72·5	0	69·2
4 oz. per bushel	70·5	0	69·2
C : Chloranil (98%)			
1 oz. per bushel	73·5	0	66·4
2 oz. per bushel	76·5	1·5	65·2
4 oz. per bushel	72·5	1·5	66·4
D : Agrosan			
1 oz. per bushel	74·5	18·5	53·2
2 oz. per bushel	76	8·5	50·4
4 oz. per bushel	73·5	6	57·2
E : Untreated	68·5	40	52

The results of a statistical analysis conducted on the pot germination figures are set out below.

Treatment.	Mean Percentage Germination.	Significantly Exceeds.	
		5% Level.	1% Level.
A. Copper carbonate	58·6	..	..
B. Thiram (50%)	69·3	D, E	D, E
C. Chloranil (98%)	66·2	D, E	E
D. Agrosan	53·8	..	..
E. Control	52·0	..	..

These results show that the thiram and chloranil dust preparations at a strength of 1 oz. per bushel were extremely effective in preventing mould growth. Agrosan at 2 oz. per bushel was fairly effective, while copper carbonate was of little value.

It is obvious that the mould was having little effect on total germination percentage in testing trays. However, the results of the fungicidal tests would indicate that where a particular preparation was effectively controlling the mould growth, germination percentage in sterile soil was slightly increased.

—*G. S. Purss.*

A Disease in Williams Hybrid Bananas Produced by Fusarium sp.

In August 1950, there appeared in a banana plantation in the Beenleigh district three Williams Hybrid plants showing symptoms similar to those produced by Panama disease in susceptible varieties. As the Williams Hybrid is a sport from the Cavendish, a variety immune to Panama disease, this occurrence was a matter of some interest.

The leaves of the affected plants were dying back from the tips but the typical bright yellow colour was absent. Slight cracks were present in the leaf sheaths at the base of the plants. Dark streaks were visible from the outside of the pseudostem and when examined internally were found to be brown "soggy" areas similar to those commonly associated with Panama disease. Many of the vascular strands in the corm tissue exhibited the reddish-brown colour typical of this disease.

A species of *Fusarium* differing from *F. oxysporum* f. *cubense* (E.F.S.) in the colour produced on rice and P.D.A. media was isolated from the diseased tissue.

In a pathogenicity test this species proved capable of attacking both the Williams Hybrid and Lady Finger varieties. Affected plants became stunted and exhibited the vascular discolouration seen in the field condition. The Cavendish variety was not affected.

F. oxysporum f. *cubense* was included in the pathogenicity trial but produced symptoms only in the Panama-susceptible Lady Finger variety.

The disease in Williams Hybrid has not recurred in the field.

—*G. S. Purss.*